REMARKS

Applicant respectfully requests that the foregoing amendments be made prior to examination of the present application.

An action on the merits is awaited.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

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By ______________________

Bernhard D. Saxe
Attorney for Applicant
Registration No. 28,665

FOLEY & LARDNER
Washington Harbour
3000 K Street, N.W., Suite 500
Washington, D.C. 20007-5109
Telephone: (202) 672-5427
Facsimile: (202) 672-5399

Version With Markings to Show Changes Made

Marked up rewritten claims:

WHAT IS CLAIMED IS:

1. (Amended) A method [Method] for inducing a red coloration by anthocyanins in plants and/or fruit which basically produce anthocyanin, which do not turn red naturally in the ripening process, or in stored fruit not colored red, the method comprising irradiating said plants or fruit [by irradiation] with an effective amount of UVB light or a mixture of UVB and white light.

2. (Amended) A method [Method] according to claim 1, wherein [in that] the irradiation based on the wattage output contains at least 10% light in UVB light wavelength ranges between 280 and 315 nm[, preferably at least 20%].

3. (Amended) A method [Method] according to claim 1, wherein said [claims 1 or 2, in that the] fruit is selected from the group consisting of apples and pears.

4. (Amended) A method [Method] according to claim 3, wherein said [in that the] apples are [preferably] selected from the varieties *Golden Delicious, Zitronenapfel, Granny Smith, and Mutsu*.

5. (Amended) A method [Method] according to claim 1, wherein said [one of the claims 1 through 4, in that the] plants and/or fruit are irradiated over a period of between 6 hours and several days[, preferably between 12 hrs and 72 hrs].

6. (Amended) A method [Method] according to claim 1, wherein said [one of the claims 1 through 5, in that the] irradiation is performed at a temperature of 0 to 30°C[, preferably at 5 to 25°C].

7. (Amended) A method [Method] according to claim 1, wherein [one of the claims 1 through 6, in that] the distance between [to] the plants and/or fruit to be irradiated and [to] the light source [source(s)] is up to 3 m[, preferably 25 to 100 cm].

8. (Amended) A method [Method] according to claim 1, wherein said [one of the claims 1 through 7, in that the] fruit is stored in a dark place after irradiation.

9. (Amended) A method [Method] according to claim 8, wherein said [in that] irradiation takes place over a period of 12 to 72 hrs, and subsequent storage in a dark place takes place for at least 2 days at 0-10°C.

10. (Amended) A method [Method] according to claim 8, wherein [claims 8 or 9, in that] after irradiation, the fruit is stored either in a ULO or CA storage.

11. (Amended) A method [Method] according to claim 1, wherein, [one of the claims 1 through 10, in that] in order to block [leave out any] anthocyanin coloration in an area having a desired [any desirable] shape, said method further comprises applying an opaque cover having [in] such [a] shape [is applied] to the plants and/or fruit with little or no coloration before the irradiation process, and then removing the [this] cover after completion of the irradiation.

12. (Amended) A plant and/or fruit of a <u>variety that does</u> not naturally <u>redden but</u> [reddening variety,] which <u>displays</u> [contains] an anthocyanin red coloration, <u>said plant and/or fruit being produced by a</u> [and is available after a] method [applied] according to [one of the claims 1 through 11] <u>claim 1</u>.

Method for inducing ~~or promoting~~ an anthocyanin coloration in plants and/or fruit which basically produce anthocyanin.

Area of the invention:

The invention relates to a method for inducing or promoting an anthocyanin coloration in plants and/or fruit which basically produce anthocyanin.

Background of the invention:

One of the most important goals of fruit production is the longest possible storage of fruit, which still looks attractive, tastes good, and is healthy. For this reason, apples as a fruit are so important at a moderate degree, because most varieties meet this requirement. In order to achieve this goal, storage of apples nowadays is done in so-called ULO storage ("ultra low oxygen"), or CA storage ("controlled atmosphere"), which contain increased carbon monoxide levels, and substantially decreased oxygen levels at a temperature of 0°C. This prevents the metabolism and ripening, and achieves a storage capability of up to 6 months or more. Prior to this method, merely the temperature was regulated, which leads to reduced storage times. To the consumer, the concentration of the red pigmentation (coloration) of the apples' skin is an important criterion for quality. The redness of the apple therefore determines the value of the apple on the market.

It is therefore considered as disadvantageous, that the redness of the fruit in a tree has always varied considerably, and the fruit growing in the shade is therefore labeled as a lower quality fruit, mainly due to its color. Additionally, there are numerous well-known and well-tasting varieties, which hardly turn red, and which are therefore less popular than they could be, such as Cox Orange. With the sales of various varieties experience clearly shows that the red color is the most important of all selection criteria. Important

varieties, which do not turn red, are Golden Delicious (Europe and worldwide), and Granny Smith, which is mostly produced in the Southern Hemisphere (New Zealand).

It is therefore the continuous goal of apple growers to produce~~, or promote~~ the red color in apples.

Although the mechanism of a pigmentation of the apple's skin cannot yet be explained in detail, it is known that adequate exposure to sunlight, but also artificial light (DE 3409796, WO 86/00492), does increase pigmentation. Additionally, it is known that chemical substances (FR 81 15845, EP 0 598 304) can also favorably influence the pigmentation of apple skin. However, the use of chemical substances is not always without risk (FR 81 15845), or does not show the desired effect in all apple varieties, and the methods using sunlight depend on their availability.

Known methods of artificial lighting generally use white light, or light simulating sunlight, that is, they attempt to supplement or replace natural sunlight.

It is also known from DE 34 09 796 how to promote anthocyanin production in fruit and plants with the use of a combination of blue and red light. The selection for the spectral range selected for this irradiation is based on the fact that two photo-chemical reactions are known for anthocyanin synthesis, namely an energy-weak red/long wave-red, reversible phytochrome controlled reaction, and an intensive irradiation reaction, which is most effective in the blue and in the long wave range of the viewable light spectrum. The phytochrome is discussed, or presumed as a photo receptor in these photo reactions involved in the production of anthocyanin.

WO 86/00492 describes a method for identifying apples, in which the apples are equipped with a lightproof mask and are then irradiated using an artificial light source

for example, a white light emitting fluorescent light source.

Nowadays, market demands require optimally reddened fruit appealing to the eye on one hand, while on the other, the market is especially critical to the use of artificial means, such as the application of chemical substances. Whereas, producing redness with the use of light for producing redness can be achieved without chemicals. However, the previously described methods, such as night interruption treatment before the harvest, are elaborate and have little effect, and the known irradiation methods using white, blue, or red light are tedious and require improvement for this reason alone. So far, a change in color of anthocyanin-colored fruit of plant parts in the sense of an increased attraction of this fruit or these plants could not be achieved.

A promotion of a natural anthocyanin coloration could, for example, be achieved on red apples (Jonagold) using UVB light and the red component of white light, as described in "PHYSIOL. PLANT 64, 323-327, 1985," or with a combination of red and blue light, as described in FR-2 542 567, whereby a treatment over a period of 30-45 days (on red Delicious, without subsequent irradiation) of the latter was required.

The presented invention is therefore based on the task of supplying a method for inducing or promoting an anthocyanin coloration in plants and/or fruit, which is characterized by an especially rapid effect, and can be easily integrated into established steps in the planting process all the way up to the sales process, while especially enabling the red coloration of plant (parts) or fruit, which normally do not develop a red color, as for instance, in so-called green apple varieties.

Abstract

Surprisingly, it was now detected that UVB light and light, which combines a mixture of white light and light from the spectral range of the UVB, not only promotes the production of anthocyanin in plants and/or fruit, but possibly even induces it. With the use of UVB light in combination with white light, the UVB contents should be higher than in sunlight, while assuming that it (median) is 2.5%.

AMENDED PAGE

~~The anthocyanin coloration according to the invention can be further promoted by irradiation using white light and additionally at least one light source emitting blue light.~~

Detailed Description of the Invention

Principally, the inventive method can be used with all anthocyanin producing structures of plants (for instance blooms and leaves), or their fruit, respectively. Anthocyanins cause a yellow, orange, red, and blue-violet as well as blue coloration, consisting of various mixed substances, which are stored in the cell plasmas. Almost all superficial cells (epidermis) of the plants' above-surface organs store anthocyanin especially well, however, they are by far not always variegated, instead they are often filled with colorless, only UV light absorbing anthocyanins. The chemical structure of the yellow, yellowish-red, and red anthocyanins is somewhat simpler than the blue ones. Some plant are unable to produce red and blue, some are unable to produce any blue anthocyanins. Anthocyanins are also the color substances responsible for the coloration of leaves (such as in dragon trees, coleus plants, and many other ornamental plants). Not all yellow/red fruit/blossoms are colored by anthocyanin. For example, red and yellow bell peppers are colored by carotinoide, which is biosynthetically produced in a completely different way.

Preferably, the inventive method for inducing ~~and promoting~~ red ~~or yellow~~ coloration (the production of red ~~or yellow~~ anthocyanins) is ~~most preferably~~ used ~~for promoting the red coloration, especially~~ in fruit.

Important fruit, which turn red ~~or yellow~~ with the aid of anthocyanin, such as apples, pears, peaches, nectarines, plums, cherries (all rose plants), blueberries, and cranberries, fall into the area of the invention. Preferably, the inventive method is used with pears and apples, but especially with apples.

~~In an especially preferred method of the invention, the production of anthocyanin is promoted in apples, which, if ripened on the tree, show a red coloration, although often~~

~~not to the desired extent. This includes *Cox Orange, Elstar, Gloster, Idared, Jonagold, and Pilot.*~~

~~In an especially preferred method of the invention,~~ [Inserted by handwriting: According to the inventive method,] the red coloration of the fruit, especially that of apples, is promoted in fruit that usually does not turn red. This is achieved by irradiation with UVB light, or a mixture of UVB light and white light, and has been successfully performed on the following apple varieties: *Golden Delicious* [inserted by handwriting: (green)], *Zitronenapfel, Granny Smith,* and *Mutsu.*

~~Due to the fact that a stronger anthocyanin production is promoted using UVB light, or a mixture of UVB light and white light, than using a mixture of white and blue light, the first two variations are preferred.~~

When using UVB light, light sources are utilized, the irradiation flow of which, ranging from 280-315 nm relative to the total irradiation flow of 100-780 nm ($\Phi_{280\text{-}315\ nm}/\Phi_{100\text{-}780\ nm}$; each measured in watts), is preferably not under 10%, or most preferably not under 20%. The currently preferred examples show a value of $\Phi_{280\text{-}315\ nm}/\Phi_{100\text{-}780\ nm}$ with at least 30%, especially with at least 45%. Higher values (i.e. at least 70%, or at least 90%) are even more beneficial in light of the energy yield. However, lamps using such a higher irradiation flow ranging from 280-315 nm, are usually expensive. Therefore, the use of more reasonably priced, commercially available UVB lamps (for instance, TL 40W/12 from the Philipps company), the value of which (approximately 57%) is upwards of 45% for $\Phi_{280\text{-}315\ nm}/\Phi_{100\text{-}780\ nm}$, can be extremely economical.

Preferably, ~~blue light sources, or~~ white light sources are used analogously, the irradiation flow of which is ($\Phi_{400\text{-}510\ nm}/\Phi_{100\text{-}780\ nm}$), or ($\Phi_{400\text{-}780\ nm}/\Phi_{100\text{-}780\ nm}$), respectively, ranging from 400-510 nm, or 400-780 nm, respectively, relative to the total irradiation flow of 100-780 nm with at least 10%, especially with at least 20% to 30%, however most preferred with at least 45%. Due to the fact that ~~blue, or~~ white light~~,~~

~~respectively,~~ with a irradiation flow of at least 70%, especially with at least 90% can be purchased at a relatively low price, working with these types of light sources is even more preferred.

~~When working with two light sources, the spectrums of which overlap, i.e. with a mixture of white and blue light, the respective irradiation flow may not be zero, and they should show the previously specified values. Alternatively, a light source can be used, if it contains an irradiation flow density appropriately supplemented in the blue range, as opposed to the white light.~~

In optimizing the procedural conditions for the irradiation, especially the number and type of the light sources used, their capacity, their arrangement, and distance relative to the fruit, the irradiation time, the temperature, and possibly a subsequent storage under cool conditions, all play a role. Generally, the irradiation with light within the wavelength range specified in detail above should be performed at such intensity, and over such a time frame, that the desired effect is achieved. The specialist can determine the parameters suitable for this in experiments, and without great effort – depending on the light sources available, and their geometric arrangement.

Typically, 1-8, preferably 1-4, but especially 2 light sources are used per light type.

The arrangement of the light sources preferably ensures that the plant(s), or the fruit is irradiated at exactly that point, at which the anthocyanin is to be produced. The arrangement of two light sources per light type above the plant(s) (fruit) is especially preferred. Preferably, both the light source and the fruit (plants) to be irradiated are arranged in an enclosure, or in a container (especially one with reflecting surfaces).

The distance between the [illegible] light source(s) and the individual plants (fruit) is preferably up to 300 cm, especially 25 to 100, whereby 60 to 80 cm is preferred. However, smaller or greater distances can be used, if the other procedural parameters (i.e. spectral irradiation flow, and capacity per lamp, irradiation time) are adjusted accordingly. For example, a greater distance between the fruit (plants) and the light source(s) can be compensated by higher capacity of the light source(s), or by a higher irradiation flow of the light source(s).

The capacity of the light source used usually lies within a range of up to 100W (20-100W), preferably 36-60W per light source. Due to heat and irradiation loss within "undesirable" spectral ranges, however, only a fraction of this capacity is usually irradiated within the "desirable" spectral section. For example, the output capacity of the commercially available UVB fluorescent lamp TL 40W/12 manufactured by Phillips lies within a range of 280-315 nm at 5.1W. When selecting a suitable light source, the fact that a higher capacity in otherwise identical procedural parameters does not automatically result in an accelerated anthocyanin production, should also be considered, because this can cause saturation effects. Wattages of 10 to 20 W/m² have been measured in some examples.

The following light intensity ranges are preferably used in the inventive method, whereby the stated values refer to the light intensity (in $\mu Es^{-1}m^2$) on the plant or the fruit, and on the wavelength range(s) of the respective light type(s).

~~Blue/white: more than 1; more preferred are more than 2; especially 20-50;~~
UVB: more than 0.5; more preferred are more than 1.0; especially 10-20;
UVB/white: more than 0.75; more preferred are more than 1.5; especially 15-20;

~~When mixing blue with white light, the ratio of light intensities (blue/white) is 1:10 to 10:1.~~ In a mixture consisting of UVB and white light, the preferred ratio is 1:20 to 10:1.

An irradiation over a time period of between 6 hrs and several days is preferred, especially preferred is 12 to 72 hrs, more preferred is 12 to 36 hrs, and the most preferred time period is 12 to 24 hrs. With irradiation of fruit, the selection of the irradiation period depends, among other considerations, on whether the fruit was freshly harvested, or already stored. Freshly harvested fruit usually reacts stronger to UVB light, possibly mixed with white light, than fruit that was stored for a longer period of time (for instance, for more than 100 days, especially if stored for more than one year), so that the end value of the red coloration can be achieved within 72 hrs. In fruit that has been stored for a longer period of time, longer irradiation times than 72 hrs may be required to achieve the end value. The suitable irradiation time period can easily be determined by observing the plants (fruit).

Temperature also influences the anthocyanin production. Typically, irradiation takes place in a climate chamber at temperatures from 5 to 25°C, preferably at 14 to 19°C (especially at 15 to 18°C), whereby the irradiation inside a climate chamber is often particularly beneficial. These temperatures influence the appearance and the taste of the apples at a minimum. An irradiation with the inventive method at 17°C has been proven successful (preferably inside of a climate chamber). Adjustment of the moisture content inside of the climate chamber is not necessary, but it can aid in maintaining the apples' "freshness."

The inventive method can also be applied to fruit still on the shrub or tree, for example, as a night interruption treatment. In the case of fruit from a tree, such as apples and pears, it is preferred to first harvest the fruit, due to economical considerations. The fruit can then be irradiated either in their fresh state, or after a freely selectable period of storage.